SUPPLEMENTARY NOTES

Family 1: The proband is a 20 year-old woman with primary amenorrhoea and absent breast development. She was born at term with a normal birthweight after an uncomplicated pregnancy. Growth and development were normal until her early to mid teen years, with no other medical problems. Her height is 140.7cm, equal to the midparental height (MPH) with an upper to lower segment ratio of 1.0 and body mass index (BMI) of 23.3 kg/m^2. Breast, pubic, and axillary hair are at Tanner Stage 1, and the rest of the physical examination is normal. Her bone age is 13 years. Pelvic ultrasonography revealed hypoplastic ovaries and uterus.

Her 24 year-old brother has a very small penis and no signs of puberty. His past medical history is also otherwise unremarkable. He is 150 cm tall (MPH 154 cm) with an upper to lower segment ratio of 1.0 and BMI 24.8 kg/m^2. Pubic and axillary hair are at Tanner stage 1. His stretched penile length is 1.5 cm (-2.5 SD for a healthy newborn male is 2.5 cm). His testes are of 1 mL volume and situated in a hypoplastic scrotum. The rest of the physical examination is normal.

Both affected siblings are of normal intelligence. They have no dysmorphic facial features, midline anomalies, retinitis pigmentosa, iris colobomata, hearing loss, hypotonia, ataxia, dementia, nor polyneuropathy. Results of Laboratory Investigation are shown in supplementary Table 2.

Their parents are known to be distant cousins. Their mother experienced menarche at age 12, and their father started shaving at age 14. Two sisters experienced menarche at 13 years of age with a regular cycle subsequently, and one 18 year-old brother has normal male physical characteristics. All are otherwise clinically well.

Family 2: The proband is a 12.8 year-old boy who presented with a small penis. He was born at term with a normal birthweight after an uncomplicated pregnancy. His past medical history is notable only for a micropenis and cryptorchidism noted at birth, for which he underwent surgical correction at 1.5 years old. His height is 145cm, consistent with the midparental centile and his BMI is 14.2 kg/m^2. Pubic and axillary hair are at Tanner stage 1. His testes are 1 ml bilaterally in the scrotum. His

stretched penile length is 2.5 cm (equal to -2.5 SD for a healthy newborn male). His bone age is 12 years.

His 15.3 year-old sister was evaluated because of absence of breast development, primary amenorrhoea, and her brother's history of hypogonadism. Her growth and development were normal until her early teen years, and she is otherwise well. Her height is consistent with her mid parental target height, and her body mass index 15.2 kg/m^2. Breasts, pubic and axillary hair are at Tanner stage 1. Her bone age is 12.5 years.

Both affected siblings show high educational achievement at school. They have no dysmorphic facial features, midline anomalies, retinitis pigmentosa, iris colobomata, hearing loss, hypotonia, ataxia, dementia, nor polyneuropathy. Results of Laboratory Investigation are shown in supplementary Table 2.

Their parents are healthy first cousins. Their mother started menstruating at age 12 and father started shaving at age 14. Their elder sister, now 16 years old, experienced menarche at 12 years of age, has a regular cycle, and is otherwise in good health. Their younger sister is 4 years old and healthy.

Family 3: The proband is a 16.2 year-old girl with primary amenorrhoea and absent breast development. She was born at term with a normal birthweight after an uncomplicated pregnancy, and grew and developed normally until her early to mid teen years. She was otherwise well. Her height is 158 cm (MPH 155.5cm) with an upper to lower segment ratio of 1.0 and BMI of 24 kg/m^2. Her breast development was at Tanner stage 1, pubic hair at stage 3, and axillary hair at stage 2. Her most recent bone age is 13 years.

Her sister is 14 years old and has the same complaints. She was born at term with a normal birthweight after an uncomplicated pregnancy, and grew and developed normally until her early to mid teen years. She is otherwise well. Her height is 145 cm with a BMI of 16.5 kg/m^2. Her bone age is 12.5 years.

Both sisters are of normal intelligence. They have no dysmorphic facial features, midline anomalies, retinitis pigmentosa, iris colobomata, hearing loss, hypotonia,

ataxia, dementia, nor polyneuropathy. Results of Laboratory Investigation are shown in supplementary Table 2.

Their parents are healthy and not knowingly related. Their mother experienced menarche at age 12 years and their father started shaving at age 14. Their 23 year-old sister experienced menarche at 12 years old and subsequently gave birth to a child. Her 15 year-old sister also experienced menarche at the age of 12. Their 11 year-old brother has 2 ml testes and a 5.5 cm normal prepubertal penis. Their remaining two sisters, aged 7 and 5 years old, are prepubertal. The youngest sibling, a 3 year-old brother, has 1.5 ml testes and 4 cm normal prepubertal penis.

Family 4: The proband is a 21.5 year-old woman with primary amenorrhoea and absent breast development. She was born at term with a birthweight of 2.3 Kg after an uncomplicated pregnancy, and grew and developed normally until her early to mid teen years. She is otherwise well. Her height is 158 cm (MPH 155cm) and BMI 19.2 kg/m^2. At 15 years old her breasts, pubic and axillary hair were all at Tanner stage 1, her bone age was 14 years, and pelvic ultrasonography showed hypoplastic ovaries and uterus. With cyclic estrogen and progesterone treatment over the subsequent 3 years, she attained Tanner stage 3 for breast, pubic and axillary hair. Her most recent laboratory evaluation off therapy has shown persisting hypogonatotropic hypogonadism, with FSH 0.05 mIU/ml, LH 0.07 mIU/ml, and estradiol 1.3 ng/dl.

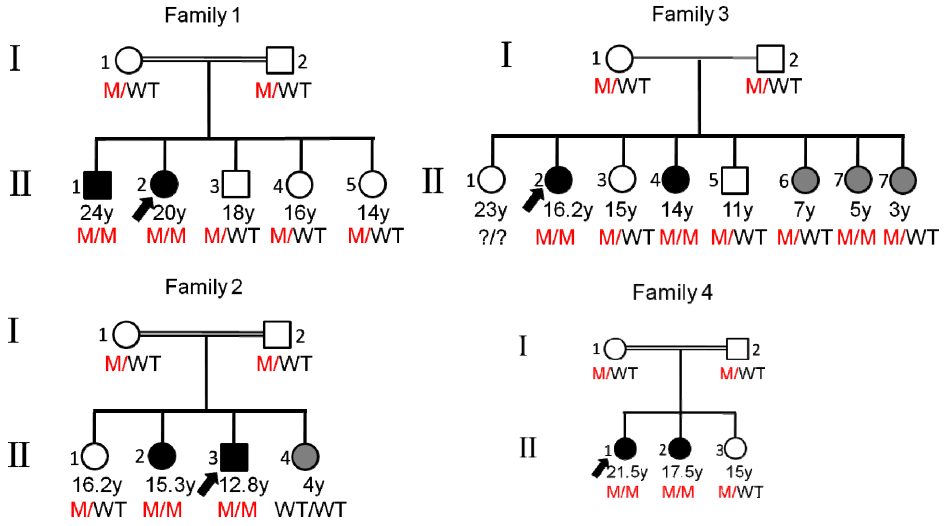
Her 17.5 year-old sister has the same complaints. She was born at term with a birth weight of 2.1 Kg after an uncomplicated pregnancy, and grew and developed normally until her early to mid teen years. She is otherwise well. At 16.5 years her height was 155 cm (MPH 159.5cm) and BMI 17.5 kg/m^2. Breasts, pubic and axillary hair were at Tanner stage 1 and her bone age was 15 years. Cyclic estrogen and progesterone treatment was subsequently given for 1 year. Her most recent laboratory testing off therapy has shown FSH 0.6 mIU/ml, LH 0.16 mIU/ml, and estradiol 1.0 ng/dl.

Both sisters are of low intelligence, and were obliged to leave school at 12 years old because of repeated failures. Evaluation with the CATTELL 2A intelligence test showed mild mental retardation with IQ scores 53 and 64. Neither affected sister has

dysmorphic facial features, midline anomalies, retinitis pigmentosa, iris colobomata, hearing loss, hypotonia, ataxia, dementia, nor polyneuropathy. Results of Laboratory Investigation are shown in supplementary Table 3.

Their parents are healthy maternal first cousins. Their mother experienced menarche at 12 years old and their father started shaving at 14 years old. Their 20 year-old sister experienced menarche at 13 years old with a regular cycle subsequently. Testing using the Wechsler intelligence test revealed a normal score of 82. She is currently attending performing well academically at school.

All pedigrees with mutation status are illustrated in the Supplementary Figure.



Supplementary Figure: Structure of Families 1-4 with genotypes indicated. Black = affected; white = unaffected; Grey = status uncertain as prepubertal girl. M = mutant, WT = wild type (TACR3 for families 1-3; TAC3 for family 4)

Family	1	1	2	2	3	3	4	4	Normal range
Member	II-2	II-1	II-3	II-2	II-2	II-4	II-1	II-3	
Sex	F	M	M	F	F	F	F	F	
Age, years	20	24	12.8	15.3	16.2	14	21.5	17.5	
Genotype	TACR3 G93D/G93D	TACR3 G93D/G93D	TACR3 P353S/P353S	TACR3 P353S/P353S	TACR3 P353S/P353S	TACR3 P353S/P353S	TAC3 M90T/M90T	TAC3 M90T/M90T	
FSH (mIU/mL)	3.23	3.03	1.15	4.9	2.06	2.0	0.05	0.41	M:1.4-18.1 F:2.5-10.2
LH (mIU/mL)	0.1	0.20	0.1	0.5	0.26	0.1	0.06	0.1	M:1.5-9.3 F:1.9-12.5
Estradiol (ng/dl)	2.6	2.4	0.59	0.52	1.0	0.5	0.1	0.02	M:0.8-3.5 F:6.3-16.5
Testosterone (ng/dl)	15.8	12.2	12.4	ND	6.0	2.6	1.12	1.12	M:350–1030 F:14-76
GnRH stimulation test[#]									See ref[28].
max FSH (mIU/mL)	12.78	11.53	6.37	16.3	7.99	8.48	2.2	2.2	
max LH (mIU/mL)	2.86	3.24	2.25	6.22	2.27	0.90	2.53	3.84	
Prolactin (pg/ml)	7.9	5.2	6.5	7.4	8.0	6.8	9.5	14	M:2.1-17.7 F:2.8-29.2
TSH (mIU/mL)	1.59	1.72	4.55	3.6	5.6	1.79	1.13	1.14	0.35-4.2
T4 (total: mcg/dl) (free: ng/dl)	10.4 (total)	9.14 (total)	1.33 (free)	1.36 (free)	1.28 (free)	1.54 (free)	1.04 (free)	0.98 (free)	Free: 0.89-1.8 Total: 4.2-13.0
Cortisol (mcg/dl)	7.0	13.5	10.7	16.4	4.6	20.5	12.3	14.7	3-25
DHEAS (mcg/dl)	ND	148	160.3	121.3	98.6	81.5	ND	ND	35-430
IGF-1 (ng/ml)	107	150	162	246	257	179	301	412	M:155–432 F:87-368
complete blood count	normal	normal	normal	normal	normal	normal	normal	normal	
biochemistry screen	normal	normal	normal	normal	normal	normal	normal	normal	

Supplementary table 1: Endocrine profile of affected individuals

SUPPLEMENTARY METHODS

Clinical Case Definition

Normosmic IHH was defined as the absence of spontaneous puberty by age 13 in girls (Tanner breast stage 1) and 14 in boys (testicular volumes of <4 ml) with prepubertal levels of sex steroids and gonadotrophins, a normal sense of smell, and bone age of 11.5 years or greater. Further affected males in multiplex families were diagnosed during infancy or childhood based on the presence of micropenis and/or cryptorchidism. All cases had otherwise normal anterior pituitary function, and no evidence of structural lesions on magnetic resonance imaging of olfactory bulbs and sulci, hypothalamus and pituitary regions. Patients with chronic systemic disease, malnutrition and extreme perturbation of bodyweight were excluded.

Functional Study of Mutant TACR3/TAC3

TACR3 cDNA in PcDNA3.1+ was purchased from the Missouri S&T cDNA Resource Center, and mutagenesis undertaken using QuickChange Site Directed Mutagenesis kits (Stratagene). After sequence verification all constructs were subcloned into pIRES2-AcGFP1 vector (Clontech) tandemly expressing green fluorescent protein (GFP) from an internal ribosomal entry site downstream from the inserted TACR3 coding sequence. HEK293A cells (QBiogene) were cultured in DMEM supplemented with 5% FCS, and were transiently transfected with the resulting expression constructs using 5μl of polyethylenimine (Sigma) per μg of DNA. 16 hours after transfection cells were seeded into glass-bottomed dishes precoated with poly-L-lysine, and experiments were undertaken after a further 24 hours with cells at 50-80% confluence. For imaging cells were loaded for 30 min at 37°C in 5 μM Fura2-AM in bath solution, containing (mM): 4.5 KCl, 138 NaCl, 4.2 NaHCO3, 1.2 NaH2PO4, 2.6 CaCl2, 1.2 MgCl, 10 HEPES (ph 7.4, NaOH) and 10 glucose.

A. TACR3 PRIMERS:

EXON	Annealing Temperature	FORWARD PRIMER	REVERSE PRIMER
1A	60	5'-CCAGCAGGGATTGCAGTATC-3'	5'-GCCAGGATGATCCAGATGAC-3'
1B	60	5'-CCAACCTCACCAACCAGTTC-3'	5'-ACTCGAGGGCTACAAATGGG-3'
2	59	5'-GCCATGATTACCATTCTACGC-3'	5'-CAACTTATTGACCACACACAAATC-3'
3	59	5'-CAACTGGCAGCATTTGAAAC-3'	5'-GATTACAGTATGTGGACAGCAGC-3'
4	58	5'-CTGTCCGTATATTGCTTCACC-3'	5'-AAAGCCTGTGCCTCTCTCAG-3'
5	59	5'-TGTGACATAAATTCTAAGAGTCTGGC-3'	5'-CCTTTCTCAATTTGACCATAGC-3'

B. TAC3 PRIMERS:

EXON	Annealing Temperature	FORWARD PRIMER	REVERSE PRIMER
2	59	5'-AAGCCAAGCTGCTGGTAATG-3'	5'-AAATGCCCTCTGACGGAC-3'
3 & 4	60	5'-GATTCAGGATGGGCTCAGG-3'	5'-GGGAGCTGGCATATTGTTTG-3'
5	59	5'-GAACAGAGACCAGAAACCCAG-3'	5'-TCCTGTCTTTCCCTCTGGTG-3'
6	58	5'-CCCAGTCTCCCAACTCTGTC-3'	5'-GCTTTAATACCTGTAGCATGGG-3'

Supplementary table 2: Primers and annealing temperatures for amplification of exons of A. TACR3 and B. TAC3